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Carotid Stiffness in Young Adults: A Life-Course Analysis of its Early Determinants

The Amsterdam Growth and Health Longitudinal Study

Isabel Ferreira, Roel J. van de Laar, Martin H. Prins, Jos W. Twisk, Coen D. Stehouwer

Abstract—Cardiovascular risk factors affecting arterial stiffness in adulthood may develop at different critical periods earlier in life. We examined whether the trajectories, from adolescence to young adulthood, of blood pressure, body fatness and fat distribution, blood lipids, cardiorespiratory fitness, and heart rate determined levels of arterial stiffness in young adults. We investigated 373 apparently healthy adults in whom cardiovascular risk factors were repeatedly examined between the ages of 13 and 36 years and carotid stiffness estimates were obtained at the age of 36 years. Differences in the mean levels and the trajectories of risk factors throughout the 24-year longitudinal period between subjects with different levels of carotid stiffness at age 36 years were analyzed with generalized estimating equations. Compared with individuals with less stiff carotid arteries, those with stiffer carotid arteries at the age of 36 years were characterized from ages 13 to 36 years by greater levels of and steeper increases in blood pressure and central fatness, independently of each other and other risk factors. These increases were already present in adolescence, preceded the development of poorer levels of blood lipids, cardiorespiratory fitness, and heart rate, which were evident during adulthood only, and explained to a great extent the deleterious association between these risk factors and carotid stiffness at the age of 36 years. Multiple and intertwined mechanisms involved in the pathogenesis of arterial stiffness have their origins in early life. Blood pressure and central fatness have a pivotal role herein and should be specifically targeted to prevent arterial stiffening and its cardiovascular sequelae. **(*Hypertension*. 2012;59:54-61.) • Online Data Supplement**

Key Words: adolescence ■ arterial stiffness ■ carotid artery ■ life-course ■ risk factors ■ young adults

Arterial stiffness is an important cause of cardiovascular disease because of its contribution to systolic hypertension, left ventricular hypertrophy, and impaired coronary perfusion.[1–3] Arterial stiffness is primarily determined by aging and mean arterial pressure (MAP),[2,4] but other risk factors (RF) may also contribute, notably body fatness and/or a central pattern of fat distribution,[5–8] impaired glucose metabolism and insulin resistance,[2] poor cardiorespiratory fitness,[9,10] and dyslipidemia.[11,12] Recently, a systematic review suggested that the contribution to arterial stiffness of RF other than blood pressure (BP) was only modest, although this evidence was derived from cross-sectional studies only.[13] Therefore, a life-course rather than a single time-point approach to the study of (early) determinants of arterial stiffness is needed.

The negligible, if any, role of RF other than BP on arterial stiffness was also emphasized in a prospective analysis conducted among men throughout middle age and older age.[14] However, some evidence suggests that arterial stiffness in adulthood has its roots early in life. Indeed, studies among the young have shown that greater levels of BP[15,16] and body fatness and/or a central pattern of fat distribution[5,15,17] measured in childhood/adolescence were associated with greater arterial stiffness in adulthood. However, how the life-course trajectories and cumulative burden of these, and also of other RF such as dyslipidemia, cardiorespiratory fitness, and resting heart rate (HR), which are all intertwined, affect arterial stiffness later in life is not known. For instance, RF affecting arterial stiffness in adulthood may develop at different critical or sensitive periods earlier in life.[18] In addition, elevations in some RF occurring early in life may accelerate over time and trigger the development of other RF, all of which may impact adversely on arterial stiffness later in life. From a preventive point of view, identifying such critical periods and triggering RF early in life is of utmost importance to inform targeted interventions with the most potential for health benefits by breaking chains of risk and enabling establishment of healthier life-course trajectories.[18]

To address these issues, we have therefore investigated in the Amsterdam Growth and Health Longitudinal Study

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(AGAHLS) the extent to which the life-course trajectories and their inter-(in)dependent associations, from ages 13 to 36 years, of blood pressure, body fatness and fat distribution, blood lipids, cardiorespiratory fitness, and resting HR determined the levels of carotid artery stiffness at age 36 years.

Subjects and Methods

Subjects and Study Design

The AGAHLS is an observational longitudinal study that started in 1977 with a group of ≈600 boys and girls from 2 secondary schools from the area of Amsterdam (The Netherlands). Its initial goal was to describe the natural development of growth, health, and lifestyle of adolescents, and to investigate longitudinal relationships between biological and lifestyle RF, as described in detail elsewhere.[19] The mean age of the subjects at the beginning of the study was 13.1±0.8 years. Since then, measurements have been obtained 2 to 8 times (up to the age of 36 years) during a 24-year follow-up period. At each measurement round, anthropometrical (body height, weight, and skinfolds [SKF]), biological (serum lipoprotein levels, BP, cardiorespiratory fitness, and HR), and lifestyle (nutritional habits, smoking behavior, daily physical activity) RF were assessed according to standard procedures[19–22] (http://hyper.ahajournals.org). In the year 2000, when the subjects' mean ages were 36.5±0.6 years, large artery properties were assessed for the first time in 373 (196 women) subjects according to guidelines for user procedures and with the use of reproducible and valid methods and devices.[1] The following carotid stiffness estimates were calculated: the distensibility coefficient (DC), the compliance coefficient (CC), and the Young's elastic modulus as described in detail elsewhere[5,8,21,23] (http://hyper.ahajournals.org).

The study was approved by the medical ethical committee of the VU University Medical Center (Amsterdam, The Netherlands). All subjects gave their written informed consent (provided by their parents when subjects were 13–16 years old).

Statistical Analyses

We used generalized estimating equations[24] to compare the trajectories and the mean levels of systolic pressure (SP), diastolic pressure (DP), and MAP, body fatness (ie, the body mass index [BMI], sum of 4 SKF) and fat distribution (SKF ratio), blood lipids (total-to-high-density lipoprotein [HDL] cholesterol, triglycerides), and physical fitness (cardiorespiratory fitness−Vo_2max, resting HR) between subjects with increasing levels of carotid stiffness at the age of 36 years. Subjects were grouped according to sex-specific tertiles (T) of each carotid stiffness estimate.

Adopting generalized estimating equations as method for data analyses allowed us to use all data available from the age of 13 to 36 years, properly adjusting for the correlation between repeated observations of the same subject and handling data from subjects with varying number and unequally time-spaced observations.[22,24] All analyses were first adjusted for sex, body height (to account for the subject's growth), and time (modeled as a categorical variable to allow departures from linearity; model 1). This model thus reflects the cumulative burden of each RF on adult carotid stiffness. Subsequently, we compared the life-course trajectories of each RF between groups with increasing gradients of carotid stiffness by adding interaction terms between group and time; results obtained were displayed graphically (smoothed line plots).[20,21] These analyses enable us to pinpoint the exact moment early in life when differences in RF between groups emerged. To analyze the extent to which the concomitant life-course of other RF explained any of the differences found between groups, analyses were further adjusted for potential confounders (ie, lifestyle variables [model 2]) and/or mediators (ie, sitting MAP, [central] body fatness, blood lipids, and physical fitness [models 3A–E]). Finally, all analyses were also adjusted for the levels of MAP at which stiffness estimates were estimated (ie, current MAP_{supine} [model 4]). Because MAP_{supine} is highly correlated with sitting MAP at age 36 years, which reflects attained levels of MAP at the end of the longitudinal period, this adjustment meant, for a great portion, removal of the effects of BP tracking.

In all generalized estimating equations analyses, an exchangeable correlation structure was used, which was deemed as the most parsimonious after examination of the interperiod correlation matrixes of the cardiovascular RF throughout the 24-year study period. All results are reported for men and women combined because no significant interactions with sex were found. Triglycerides levels, which were positively skewed, were log-transformed before all analyses.

Statistical significance was set at $P<0.05$. All analyses were performed with the use of the STATA software package version 11 (STATA Corp, College Station, TX).

Results

From the lowest (T1) to the highest tertiles (T3), subjects' mean±SD levels of the carotid DC (in 10^{-3}/kPa) were 20.3±2.2, 26.2±1.6, and 33.4±4.1, of the carotid CC (in mm^2/kPa) were 0.72±0.12, 0.97±0.09, 1.28±0.20, and of the carotid Young's elastic modulus (in 10^3kPa) were 0.32±0.05, 0.43±0.03, and 0.58±0.09 (P for linear trend <0.001 for all). Differences in the carotid DC and CC between subjects in the highest vs lowest tertiles were equivalent to values observed in the course of one decade of aging;[25] groups herein defined as having stiffer arteries (ie, in T1 for DC and CC or in T3 for Young's elastic modulus) and less stiff arteries (in T3 for DC and CC or in T1 for Young's elastic modulus) translate into potentially clinical relevant differences in carotid stiffness levels.

Table 1 shows the general characteristics of the study population throughout the longitudinal period stratified by levels of subjects' carotid DC at age 36 years. All data shown are those of groups defined on the basis of this stiffness estimate; qualitatively similar findings were found when groups were defined on the basis of the carotid CC or Young's elastic modulus instead (Supplemental Table S1, http://hyper.ahajournals.org).

Trajectories and Cumulative Burden of Blood Pressure

Subjects with stiffer arteries (ie, in T1 of the carotid DC) had on average and throughout the whole longitudinal period (in mm Hg [95% CI]) 5.3 (3.9–6.8), 4.7 (2.6–6.8), and 5.7 (4.3–7.1) greater levels of MAP, SP, and DP, respectively, than those with less stiff arteries (T3; Table 2, model 1). Importantly, these differences were not constant over time, and although being already present during adolescence (eg, at age 14 years: 3.5 [1.5–5.5] for MAP, 2.6 [0.1–5.1] for SP, and 4.0 [1.6–6.3] for DP), were further amplified from this age onward and more strongly so in subjects with stiffer arteries (rate of BP increase, in mm Hg/year [95% CI]: 0.51 [0.43–0.59] for MAP, 0.31 [0.21–0.40] for SP, and 0.61 [0.52–0.70] for DP) than with less stiff arteries (0.28 [0.20–0.37] for MAP, 0.02 [−0.08–0.13] for SP, and 0.41 [0.32–0.51] for DP; Figure A–C). These steeper increases in BP resulted in differences between the 2 groups that were ≈2.5-fold greater at age 36 years (9.0 [6.5–11.4], 8.7 [5.5–11.8], and 9.1 [6.7–11.6], for MAP, SP, and DP, respectively) vs age 14 years. Adjustment for lifestyle variables did not materially change the mean differences over time between groups (model 2), and further adjustment for other RF attenuated the differences in BP, mainly because of central fatness (model 3B), which nevertheless remained significant. Further adjustment for current MAP_{supine} attenuated the differences in BP

Table 1. Characteristics of the Study Population (n=373)* Throughout the 24-Year Longitudinal Period by Levels of Carotid Stiffness at Age 36 Years

		Age (y)							
Characteristics	Group	13	14	15	16	21	27	32	36
Blood pressure									
Mean arterial pressure, mm Hg	T1	92.7±6.6	92.5±6.0	90.5±6.0	93.3±6.2	96.1±8.3	98.6±8.5	102.2±9.8	105.0±10.6
	T2	91.3±7.6	91.7±7.5	90.0±7.6	91.5±7.8	95.8±8.8	97.6±8.7	99.0±8.4	100.0±10.2
	T3	91.4±6.6	90.5±5.8	89.5±6.2	89.5±7.3	93.5±6.1	94.3±7.2	97.7±8.2	97.0±10.9
Systolic pressure, mm Hg	T1	124.8±8.7	123.2±8.8	125.0±9.5	126.6±9.5	128.9±11.0	130.2±11.6	131.4±12.9	135.4±13.4
	T2	124.5±9.5	123.3±9.3	125.1±10.3	125.5±11.6	129.3±12.6	129.4±12.6	128.7±12.0	130.0±13.7
	T3	124.7±10.0	123.5±9.8	125.4±10.1	125.6±10.9	127.9±9.9	128.2±11.5	128.9±12.3	128.3±15.0
Diastolic pressure, mm Hg	T1	76.7±7.3	77.2±7.0	73.3±7.8	76.6±7.0	79.7±8.6	82.8±8.7	87.6±9.4	89.8±10.5
	T2	74.8±8.7	75.9±8.6	72.4±9.1	74.5±8.2	79.1±9.4	81.7±8.6	84.2±8.1	85.2±9.4
	T3	74.8±7.2	73.9±6.3	71.6±6.9	71.4±8.6	76.3±5.7	77.4±7.2	82.2±8.0	81.3±10.2
Prehypertension/ hypertension, %	T1		2.5/2.5			25.8/11.3	22.6/22.6	25.9/34.8	32.3/37.9
	T2		3.3/3.3			20.8/18.8	23.5/17.6	32.2/19.1	29.6/22.4
	T3		2.5/2.5			25.7/5.7	19.4/13.9	20.8/20.0	27.4/13.7
Body fatness/fat distribution									
Body mass index, kg/m^2	T1	18.0±2.1	18.5±2.3	19.4±2.4	20.1±2.5	21.5±2.5	22.5±2.7	24.0±3.3	24.9±3.6
	T2	17.6±1.6	18.2±1.8	19.0±1.8	19.6±1.7	21.2±1.9	22.2±1.9	23.3±2.7	23.9±2.7
	T3	17.5±1.7	18.7±2.0	19.3±1.9	19.8±1.7	21.4±2.1	21.9±2.2	22.8±2.7	23.5±2.9
Overweight/obesity, %	T1		4.1/0			8.1/0	19.4/0	29.5/3.6	37.1/8.9
	T2		0.8/0			2.1/0	9.8/0	18.0/1.7	28.8/1.6
	T3		2.5/0			2.9/0	8.3/0	10.0/2.5	21.8/3.2
Sum of 4 skinfolds, mm†	T1	33.9±14.3	33.9±15.1	37.0±16.8	40.9±19.6	47.2±19.4	46.0±18.9	52.5±21.8	56.6±19.5
	T2	31.1±10.9	33.0±13.2	33.6±13.6	37.8±14.3	43.2±14.4	40.6±13.9	46.5±17.7	50.0±16.1
	T3	30.8±9.7	33.5±13.7	35.2±14.2	37.1±13.7	42.7±16.7	36.5±11.7	43.3±17.0	48.0±17.7
Skinfold ratio‡	T1	0.50±0.06	0.52±0.05	0.54±0.06	0.55±0.06	0.58±0.07	0.57±0.09	0.57±0.09	0.59±0.10
	T2	0.49±0.06	0.51±0.06	0.53±0.06	0.54±0.06	0.57±0.07	0.56±0.08	0.57±0.09	0.57±0.10
	T3	0.48±0.06	0.50±0.06	0.52±0.06	0.54±0.06	0.57±0.09	0.56±0.09	0.55±0.09	0.55±0.10
Blood lipids									
Total-to-high-density lipoprotein cholesterol ratio	T1	3.1±0.7	3.1±0.6	3.3±0.6	3.1±0.7	3.7±0.8	3.7±1.0	3.8±1.2	4.1±1.4
	T2	3.2±0.7	3.2±0.7	3.5±0.8	3.4±0.8	3.8±0.8	3.9±0.9	3.8±1.2	3.9±1.3
	T3	3.1±0.7	3.1±0.7	3.4±0.9	3.2±0.7	3.8±1.1	3.7±1.3	3.5±1.1	3.5±1.2
Triglycerides, mmol/L	T1	. . .	. . .	. . .	. . .	0.8 (0.5–1.2)	0.8 (0.7–1.2)	1.0 (0.7–1.4)	1.2 (0.8–1.6)
	T2	. . .	. . .	. . .	. . .	0.8 (0.6–1.1)	1.1 (0.9–1.4)	1.0 (0.7–1.3)	1.1 (0.8–1.6)
	T3	. . .	. . .	. . .	. . .	0.7 (0.5–1.0)	0.8 (0.7–1.1)	0.9 (0.7–1.2)	0.9 (0.7–1.2)
Physical fitness									
VO_2max, mL/min per kg^{FFM}	T1	68.9±6.7	68.6±5.8	66.4±6.5	65.9±6.6	59.5±6.6	55.8±6.1	55.4±7.2	60.1±8.2
	T2	70.3±6.6	68.7±6.4	67.6±5.1	66.3±6.3	60.9±5.7	56.9±6.1	56.6±8.0	59.6±8.7
	T3	69.3±6.1	69.4±6.7	67.2±5.2	66.4±6.9	58.9±6.2	57.4±7.2	57.2±6.9	61.9±8.2
Resting heart rate, bpm	T1	85.4±14.7	80.9±13.7	81.1±15.3	77.5±14.5	74.4±14.8	76.4±12.9	79.0±13.7	74.4±11.5
	T2	81.9±14.1	79.6±13.6	77.5±15.5	75.4±12.7	71.8±11.7	70.2±11.8	74.5±13.5	69.7±9.4
	T3	82.9±14.0	77.2±12.3	76.4±12.5	73.9±14.4	69.5±12.5	67.5±12.0	72.8±13.8	69.4±11.2
Lifestyle									
Total energy intake, 10^3 kcal/d	T1	2.47±0.54	2.46±0.59	2.50±0.61	2.47±0.68	2.50±0.71	2.31±0.55	2.47±0.64	2.54±0.60
	T2	2.44±0.56	2.49±0.54	2.64±0.66	2.60±0.64	2.67±0.73	2.54±0.64	2.57±0.72	2.59±0.73
	T3	2.48±0.54	2.61±0.64	2.64±0.77	2.60±0.75	2.76±0.76	2.67±0.73	2.75±0.74	2.74±0.74
Total physical activity, 10^3 metabolic equivalent/wk	T1	4.43±1.96	4.15±1.67	3.73±1.73	3.50±1.74	3.20±2.24	2.94±2.07	3.31±2.45	4.58±2.79
	T2	4.44±1.90	3.75±1.54	3.77±1.60	3.45±1.36	3.32±1.91	3.36±2.24	3.43±2.48	4.87±3.24
	T3	4.38±1.56	4.11±1.64	3.60±1.49	3.62±1.51	3.38±2.06	2.61±1.47	3.50±1.96	5.19±3.70

(*Continued*)

Table 1. Continued

Characteristics	Group	Age (y)							
		13	14	15	16	21	27	32	36
Smoking, %	T1	3.4	10.8	10.7	25.3	31.1	19.4	20.5	21.8
	T2	1.2	10.7	16.4	9.1	20.8	25.5	21.4	24.2
	T3	0.0	11.8	15.3	17.8	40.0	38.9	18.8	24.4
Alcohol consumption, %	T1	16.3	18.9	36.0	50.6	71.0	74.2	83.2	81.8
	T2	13.4	18.4	39.2	50.0	66.7	72.5	77.4	82.9
	T3	10.1	7.8	22.4	41.7	68.6	69.4	80.5	81.5

Data are means±SD or percentages or median (interquartile range).

For metabolic equivalents, 1 metabolic equivalent represents the energy expended in 1 min by a person at rest.

*Exact n differs at each time point and per variable because of missing observations.

†Biceps, triceps, subscapular and suprailiac.

‡The ratio of the subscapular+suprailiac to the biceps+triceps+subscapular+suprailiac skinfolds.

Tertiles (T) represent groups with increasing levels of the carotid distensibility coefficient at age 36 years: T1, with stiffer arteries; T2, with intermediate stiff arteries; T3, with less stiff arteries. FFM indicates free fat mass.

between groups considerably, although the differences in MAP and DP remained statistically significant (model 4).

Trajectories and Cumulative Burden of Fatness and Fat Distribution

Subjects with stiffer arteries at age 36 years had greater mean levels of BMI (1.06 kg/m^2; 95% CI, 0.47–1.65), sum of 4 SKF (ΣSKF, 6.1 mm; 95% CI, 2.9–9.4), and SKF ratio (*10; 0.36; 95% CI, 0.22–0.51) over the whole longitudinal period, as compared with those with less stiff arteries (Table 2, model 1). Subjects with stiffer arteries also had steeper increases in these estimates from adolescence to age 36 years (Figure D–F). For instance, BMI levels differed significantly from those with less stiff arteries already at age 15 years (0.71 kg/m^2; 95% CI, 0.04–1.39), and this difference was approximately twice as much at age 36 years (1.59 kg/m^2; 95% CI, 0.76–2.42). Similarly, the differences in SKF ratio (*10), which were already present at age 13 years (0.26; 95% CI, 0.08–0.43) increased even more thereafter, being ≈2-fold greater at age 36 years (0.46; 95% CI, 0.28–0.65). Adjustments for lifestyle variables did not materially change the mean differences over time between groups (model 2), but further adjustment for other RF (model 3), particularly MAP (model 3A) and total-to-HDL cholesterol ratio (model 3B), attenuated the differences in BMI and ΣSKF considerably. Noteworthy, the differences in the SKF ratio were affected to a lesser extent by these adjustments (models 3) and remained significant, even when these included current MAP_{supine} (model 4).

Trajectories and Cumulative Burden of Blood Lipids and Physical Fitness

Subjects with stiffer arteries, as compared with those with less stiff arteries, had on average 0.37 (95% CI, 0.15–0.56)

Table 2. Mean Differences in Blood Pressure and Body Fatness Throughout the 24-Year Longitudinal Period Between Subjects With Stiffer vs Less Stiff Carotid Arteries at Age of 36 Years

Model	Adjustments	Mean Arterial Pressure (mm Hg)		Systolic Pressure (mm Hg)		Diastolic Pressure (mm Hg)		Body Mass Index (kg/m^2)		Sum of 4 Skinfolds (mm)		Skinfold Ratio (*10)	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
1	Sex, height and time	5.3‡	3.9; 6.8	4.7‡	2.6; 6.8	5.7‡	4.3; 7.1	1.06‡	0.47; 1.65	6.1‡	2.9; 9.4	0.36‡	0.22; 0.51
2	Model 1+lifestyle variables§	5.2‡	3.7; 6.6	4.5‡	2.4; 6.6	5.5‡	4.1; 6.9	1.08†	0.46; 1.70	6.2‡	2.9; 9.6	0.34‡	0.20; 0.49
3A	Model 2+mean arterial pressure	. . .	. . .	. . .	. . .	. . .	. . .	0.70*	0.10; 1.30	3.9*	0.7; 7.2	0.28‡	0.14; 0.43
3B	Model 2+skinfolds ratio	4.6‡	3.1; 6.0	3.7†	1.6; 5.8	5.0‡	3.6; 6.5	. . .	. . .	. . .	. . .	. . .	. . .
3C	Model 2+total-to-hihg-density lipoprotein cholesterol ratio	4.7‡	3.2; 6.1	4.0‡	1.9; 6.1	5.1‡	3.7; 6.4	0.77*	0.18; 1.36	4.3†	1.1; 7.5	0.27‡	0.14; 0.41
3D	Model 2+VO_2max	5.1‡	3.7; 6.6	4.5‡	2.4; 6.5	5.5‡	4.1; 6.8	1.02†	0.41; 1.63	5.9†	2.6; 9.2	0.34‡	0.20; 0.48
3E	Model 2+resting heart rate	4.9‡	3.5; 6.3	4.1‡	2.1; 6.2	5.3‡	3.9; 6.6	1.05†	0.43; 1.67	5.8†	2.5; 9.1	0.34‡	0.20; 0.49
3	Model 2+all risk factors in models 3A–E	4.1‡	2.6; 5.5	3.0†	0.9; 5.1	4.6‡	3.2; 6.0	0.46	−0.11; 1.03	2.4	−0.7; 5.5	0.25‡	0.11; 0.38
4	Model 3+current MAP_{supine}	1.7*	0.3; 3.1	0.0	−2.2; 2.2	2.6‡	1.2; 3.9	0.19	−0.42; 0.80	1.1	−2.1; 4.2	0.29‡	0.15; 0.43

β values are longitudinal regression coefficients and indicate mean differences over time between subjects with stiffer (lowest tertile) vs less stiff (highest tertile of the carotid distensibility coefficient) for each risk factor listed in column headings. Each row indicates such differences after successive adjustments for the (longitudinal) levels of the variables specified by each model.

*P<0.05; †P<0.01; ‡P<0.001.

§Total energy intake, physical activity, smoking, and alcohol consumption status.

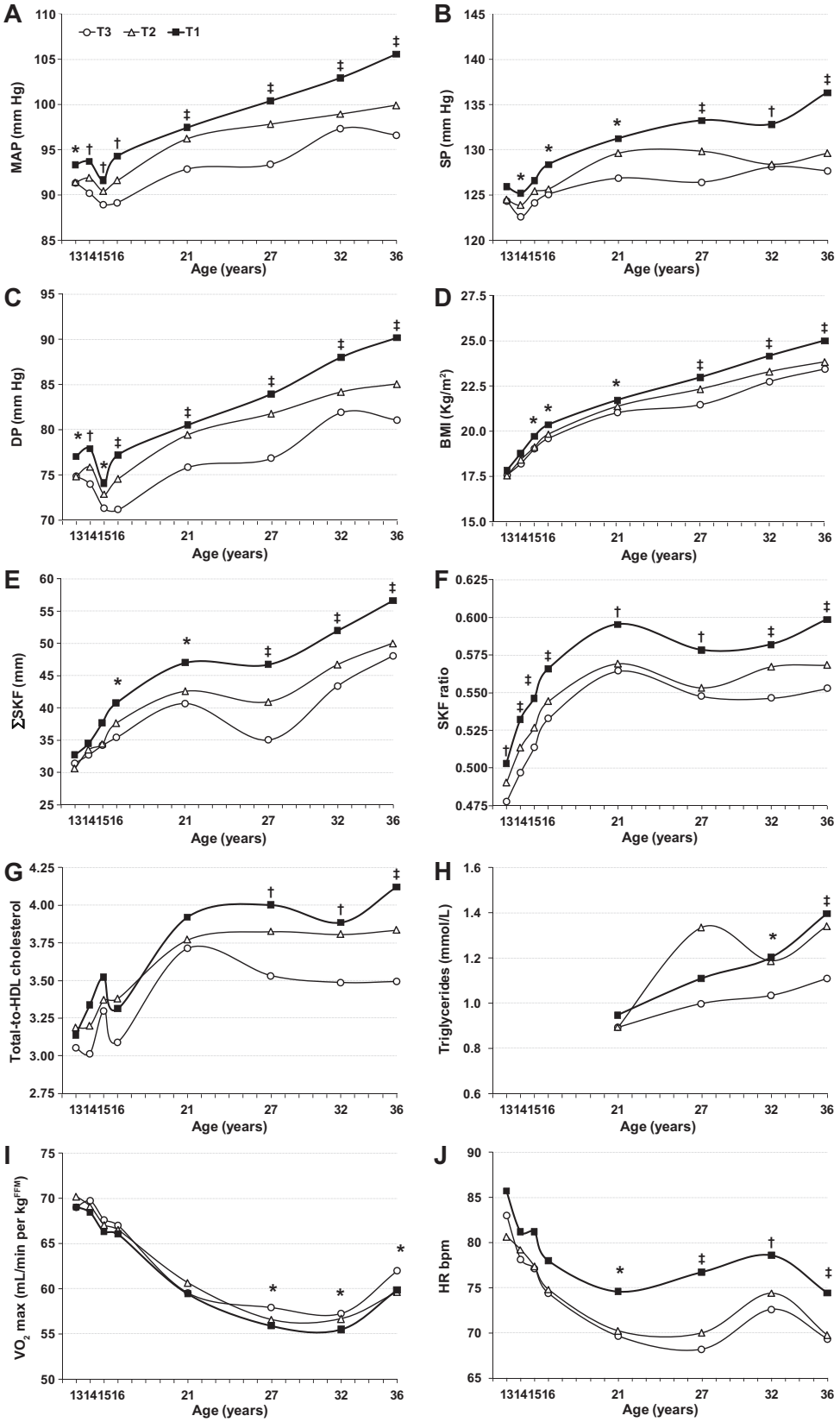

Figure. Comparison of the life-course trajectories of potential determinants of arterial stiffness between subjects with stiffer (ie, lowest tertile [T1]) vs less stiff carotid arteries (highest tertile [T3]) at age 36 years: (**A**) mean arterial pressure (MAP); (**B**) systolic pressure (SP); (**C**) diastolic pressure (DP); (**D**) body mass index (BMI); (**E**) sum of four skinfolds (ΣSKF); (**F**) SKF ratio; (**G**) total-to-high-density lipoprotein (HDL) cholesterol ratio; (**H**) triglycerides; (**I**) cardiopulmonary fitness (Vo_2max); and (**J**) resting heart rate (HR). Lines for the middle tertile (T2) indicate patterns for subjects with intermediate levels of carotid stiffness. All data are adjusted for sex and height; values for triglycerides are geometric means. *$P<0.05$, †$P<0.01$, and ‡$P<0.001$ for comparisons between subjects with stiffer vs less stiff carotid arteries.

greater levels of total-to-HDL cholesterol ratio and 1.08-times (95% CI, 1.03–1.12) greater levels of triglycerides throughout the longitudinal period. The differences in total-to-HDL cholesterol ratio emerged after adolescence only, becoming significant from the age of 27 years onward (Figure G); the differences in triglycerides, which were assessed during young adulthood only, were present at ages 32 and 36 years (Figure H). The mean differences over time in both total-to-HDL cholesterol and triglycerides were independent of lifestyle variables (model 2) but were greatly reduced after adjustments for other risk factors, particularly MAP and the SKF ratio (models 3A and 3B; Supplemental Table S2; http://hyper.ahajournals.org).

Subjects with stiffer arteries, as compared with those with less stiff arteries, had on average 1.39 mL/min/kg^{FFM} (95% CI, −2.58 to −0.19) lower levels of Vo_2max and 4.7 bpm (95% CI, 2.3–7.1) higher levels of HR throughout the whole longitudinal period. Although Vo_2max and HR decreased from adolescence to age 36 years in all subjects, these trajectories were more adverse in individuals with stiffer arteries. However, significant differences between groups were only observed during adulthood (at age 27 years and onward for Vo_2max, Figure I; and at age 21 years and onward for HR, Figure J). Mean differences over time between groups were only slightly attenuated after adjustments for lifestyle RF (model 2), but more markedly so after further adjustments for the other RF (model 3; Supplemental Table S2; http://hyper.ahajournals.org).

Discussion

We investigated the trajectories, from adolescence to adulthood, of potential determinants of carotid stiffness. We show that as compared with individuals with less stiff arteries at age 36 years, those with stiffer arteries were characterized, from ages 13 to 36 years, by greater levels of and steeper increases in BP and central fatness, independently of each other and of other RF. These increases were already present in adolescence, preceded the development of poorer levels of blood lipids, cardiorespiratory fitness, and heart rate, which were evident during adulthood only, and explained, to great extent, the deleterious association between these RF and carotid stiffness at age 36 years. This is the first study to investigate and tease apart the relative role of several potential determinants of arterial stiffness with a longitudinal design. Our findings support the view of adolescence as a critical period for the development of elevated BP, mainly DP, and (central) fatness and its associated cardiovascular morbidities later in life.[26–28]

In agreement with previous observations,[15–17] we show that subjects with stiffer arteries in adulthood were characterized by greater levels of BP early in life. Given the great dependence of arterial stiffness on the levels of transmural pressure at which they are measured, and given the phenomenon of BP tracking throughout age, the marked attenuation in BP differences after adjustment for current MAP_{supine} was largely expected. Increased arterial stiffness is primarily determined by the properties of the extracellular matrix (elastin, collagen) and vascular smooth muscular cell function.[1,2,29] These properties are strongly affected by lifelong BP.[4,13] Our longitudinal approach enabled us to pinpoint adolescence as the period early in life when increases in predominantly DP, but also in SP pressure, and thus MAP, may be linked to greater arterial stiffness later in life. The observation of DP as a major determinant of arterial stiffness may reflect the phenomenon of "downstream" increase in resistance at the level of the arterioles, leading to an "upstream" increase in transmural pressure, resulting in both structural and functional disruption of the arterial pressure load-bearing elastin–collagen network within the media layer, and thus greater arterial stiffness.[4,29] Our data thus also are consistent with the subtypes of elevated SP and DP or isolated diastolic hypertension that typically characterize young adults. Still, with aging, increased arterial stiffness may affect the BP–arterial stiffness relationship, such that after age ≈50 to 60 years[4] another phenotype becomes more prevalent, that of isolated systolic hypertension.

Another key finding of the present study was that the levels of central rather than of total body fatness during adolescence impacted on arterial stiffness in adulthood. Body fatness, particularly central fatness, is a well-recognized correlate of arterial stiffness,[5–8] even though the mechanisms linking the two are incompletely understood.[30] The impact of total or central body fatness on other cardiovascular RF and their clustering may constitute one such mechanism.[30] We have previously shown in this cohort that increases in total and central body fatness from adolescence to young adulthood were critical for the development of the metabolic syndrome in adulthood,[20] which, in turn, was associated with greater arterial stiffness.[23] In the present study, we show longitudinally that the levels of mainly MAP and dyslipidemia and to a less extent of physical fitness could explain a great part of the association between total body fatness (up to ≈60%), but less so of the association between central body fatness and arterial stiffness (≈25% only). This could be appreciated by the change in the magnitude of the differences in BMI or the ΣSKF and the SKF ratio after adjustment for those risk factors (ie, model 3 vs model 2 in Table 2). Other (central) adiposity-related factors thus also may contribute to arterial stiffness. These may include adrenergic (sympathetic overactivity) and metabolic (eg, insulin resistance, hypoadiponectinemia, hyperleptinemia, proinflammatory cytokines) pathobiological mechanisms.[30] Measures of these potential explanatory mechanisms were not assessed throughout the whole longitudinal period covered in this study, however.

Noteworthy, subjects with stiffer arteries in adulthood were characterized not only by greater levels of BP and central fatness extending back to early age, which is supportive of tracking, but also by steeper increases in BP (mainly DP) and (central) fatness, particularly during young adulthood, supportive of a "horse-racing" phenomenon surrounding these risk factors.[31] These observations may have important implications for prevention. Tracking, ie, the stability of rank of an individual in the RF distribution over time has bearing in the early detection of subjects at risk in the sense that RF levels in young adulthood will be good predictors of their levels later in life.[22] Horse-racing suggests that the rate of change of these RF, in addition to their absolute levels at any given point in time, may contribute to the identification of subjects

at risk and likely to benefit from preventive measures, and thus should be monitored.[32] In support of this, in the present cohort the prevalence of (pre)hypertension or overweight/obesity during adolescence was very low and, per se, not predictive of significantly greater carotid stiffness (lowest versus other tertiles) later in life (OR, 0.85; 95% CI, 0.32–3.26; and OR, 2.54; 95% CI, 0.67–9.55, respectively). Instead, increases in BP and (central) fatness observed between adolescence and young adulthood, even when occurring within the range of values below those commonly used as indicative or risk, seemed to be setting the grounds for greater arterial stiffness later in life. In this respect, and on the basis of these RF, our data suggest that subjects with less stiff carotid arteries were those who, between adolescence and young adulthood, had relatively lesser increases in SP and DP (barely exceeding the mean values of 130 and 85 mm Hg, respectively, at age 36 years), BMI (not exceeding the mean value of 24 kg/m^2 at age 36 years), and SKF ratio $<$0.57 throughout the adult period (for age-specific references please see Figure); these values could be used as references for a healthy profile within the age periods as examined herein. However, a note of caution is warranted here. The magnitude of the differences found in our study between individuals with stiffer vs less stiffness carotid arteries were small relative to the observed variability in the lifelong levels of the RF examined. Larger studies are needed to ascertain with more certainty the limits of such a healthy or desirable risk profile in young age.

Differences in total-to-HDL cholesterol ratio, triglycerides, cardiorespiratory fitness, and resting HR between subjects with stiffer vs less stiff carotid arteries in adulthood were only visible during adulthood. However, the deleterious impact of each of these RF on arterial stiffness was, to a great extent, explained by concomitant trajectories of the other RF, mainly the increases in BP and (the accentuation of a central pattern of) body fatness, which actually preceded and thus may have triggered the former.

There are limitations to the present study that need to be addressed. First, our findings were confined to subjects in whom complete data for arterial properties were obtained during the follow-up examination in the year 2000. However, levels of BP, total and central fatness, blood lipids, cardiorespiratory fitness, and HR in these subjects did not differ, at any earlier time point, from those subjects who dropped-out (data not shown); this indicates that selection bias did not threaten the validity of our findings. Second, the trajectories of the RF reported herein refer to subjects who were adolescents in the late 1970s. Given the current obesity epidemic in youth, it is possible that the critical periods identified herein may have shifted to even earlier ages. From an etiologic point of view, this does not hinder the validity of our findings; in fact, it just emphasizes the need for public health interventions targeting young people.[26,27] Third, although we have adjusted our analyses extensively for several potential confounders, we cannot fully exclude the possibility of residual confounding. Fourth, although the use of local pulse pressure estimated by calibration of the distension waveforms[25] instead of brachial pulse pressure for the calculations of the carotid stiffness estimates constitutes a strength to our study, this method still may not optimally reflect the level of pulse pressure at the level of the carotid artery. Finally, given its observational design, this study cannot prove causality; furthermore, because carotid stiffness levels were measured at age 36 years only, we cannot rule out the possibility of reverse causality (eg, that subjects with stiffer arteries at any earlier time point may have been less prone to adopt healthier lifestyles, which could lead to better cardiovascular risk profiles). However, we deemed this less likely because the young subjects were, throughout the whole longitudinal period, unaware of and asymptomatic with regard to their stiffness levels.

Perspectives

Our findings support the existence of multiple and intertwined mechanisms in the pathogenesis of arterial stiffness that have their origins in early life. BP and a central pattern of body fatness have a pivotal role herein. Efforts to prevent arterial stiffening and its cardiovascular sequelae thus most usefully may be targeted at the prevention of increases in BP and central fatness starting during early age.

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Disclosures

None.

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ONLINE SUPPLEMENT

CAROTID STIFFNESS IN YOUNG ADULTS: A LIFE-COURSE ANALYSIS OF ITS EARLY DETERMINANTS

The Amsterdam Growth and Health Longitudinal Study

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Supplementary methods description

Supplementary Tables S1 and S2

METHODS

Longitudinal measurements of cardiovascular risk factors

Blood pressure was measured twice with a sphygmomanometer (Speidl-Keller, Franken & Itallie, Amsterdam, The Netherlands) and a standard pressure cuff, after subjects had rested in a sitting position for at least 5 min. The lowest value of the systolic (SP) and diastolic blood pressure (DP) values thus obtained were recorded and used in the analyses.[1] Mean arterial pressure (MAP), throughout the longitudinal period, was calculated as [(2*DP)+SP]/3. Prevalence of pre-hypertension or hypertension was defined: using sex, age and height-specific cut-off values for SP and/or DP according to the Task Force for Blood pressure in Children criteria and during adolescence (considered present if exceeding those cut-off values in at least 3 moments during this period);[2] if SP/DP≥130/85 or ≥140/90 during adulthood.[3]

Anthropometric measures included standing height, body weight, and biceps, triceps, subscapular and suprailiac skinfolds and were performed by trained observers. We calculated, as indicators of total body fatness, the body mass index (BMI; in kg/m^2) and the sum of the thickness of the four skinfolds (ΣSKF, in mm); the ratio of the subscapular + suprailiac skinfolds to the ΣSKF (SKF ratio) was used as an estimate of central fat distribution.[1,4-7] Prevalence of overweight or obesity was defined using age and sex-specific cut-off values for BMI according to the International Obesity Task Force criteria during adolescence, and as BMI≥25 or ≥30 kg/m^2, respectively, during adulthood.[8]

Total and HDL-cholesterol and triglycerides, the latter from the age of 21 onwards only, were measured in non-fasting blood samples (10 mL) drawn from the antecubital vein with the use of enzymatic techniques (Roche Diagnostics, Mannheim, Germany); throughout the years, external quality control of these measures took place with target samples from a World Health Organization reference laboratory.[1,9,10]

Throughout the years cardiorespiratory fitness was measured in the same laboratory with the same protocol and equipment: a maximal running test on a treadmill (Quinton, Bothel, Washington, USA, model 18-54) with direct measurements of oxygen uptake (Ergoanalyzer, Jager, Bunnik, The Netherlands). Subjects were instructed to run at a constant speed of 8 km/h while the slope of the treadmill increased every 2 minutes in a stepwise fashion, and were encouraged to continue running to their maximum. Maximal oxygen uptake (VO_2max) expressed by kg of fat-free mass (i.e. mL/min/kg^{FFM}) was used in the analyses as a measure of cardiorespiratory fitness.[11] FFM was derived by subtracting fat mass, which was calculated from skinfold thickness according to age and sex-specific equations [12,13] from total body weight. Resting heart rate (HR) was measured telemetrically (Telecust 36 and Sirecust BS1, Siemens, Amsterdam, The Netherlands) as the mean value from 15 R-R intervals in the last 15 seconds of the minute, after subjects had been sitting on a chair for 5 minutes.

Information on habitual physical activity levels, total energy intake, alcohol consumption and smoking behavior were assessed by means of interviews and questionnaires.[14-16]

Arterial stiffness

Briefly, all subjects had abstained from smoking and caffeine-containing beverages on the day the measurements were performed. Measurements took place after subjects had been resting in a supine position for 15 min in a quiet temperature-controlled room. Properties of the right common carotid artery (10 mm proximal to the beginning of the bulb) were obtained by two trained vascular sonographers with the use of an ultrasound scanner equipped with a 7.5-MHz linear array probe (Pie Medical, Maastricht, The Netherlands). The ultrasound scanner was connected to a personal computer equipped with an acquisition system and a vessel wall movement detector software system (Wall Track System 2, Pie Medical, Maastricht, The

Netherlands). This integrated device enabled measurements of arterial diameter (D), distension (ΔD), and intima-media thickness (IMT) as described in detail elsewhere.[17]

Throughout the entire period of ultrasound imaging, systolic (SP), diastolic (DP) and mean arterial pressure (MAP_{supine}) were assessed in the left arm at 5 minutes intervals with an oscillometric device (Colin Press-Mate, model BP-8800, Komaki-City, Japan). Brachial artery pulse pressure (PP) was defined SP – DP, and PP at the level of the common carotid artery was calculated by calibration of the diameter distension waveforms obtained at the brachial and carotid arteries.[18] The mean D, ΔD, IMT and local PP of 3 consecutive measurements (each including 3 to 7 heart beats) were used to estimate the carotid distensibility (DC) and compliance (CC) coefficients, and the Young's elastic modulus (YEM) as follows:[4,5,17]

$DC=(2\Delta D \cdot D+\Delta D^2)/(PP \cdot D^2)$ in 10^{-3}/kPa

$CC=\pi \cdot (2D \cdot \Delta D+\Delta D^2)/4PP$ in mm^2/kPa

$YEM=D/(IMT \cdot DC)$ in $10^3 \cdot$kPa

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Table S1. Mean differences in blood pressure and body fatness, throughout the 24-year longitudinal period, between subjects with stiffer *vs.* less stiff carotid arteries at the age of 36, as defined on the basis the carotid DC, CC or YEM.

	Mean arterial pressure (mmHg)						Systolic pressure (mmHg)						Diastolic pressure (mmHg)					
	T1 vs. T3		T1 vs. T3		T3 vs. T1		T1 vs. T3		T1 vs. T3		T3 vs. T1		T1 vs. T3		T1 vs. T3		T3 vs. T1	
Model	DC		CC		YEM		DC		CC		YEM		DC		CC		YEM	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
1	5.3‡	3.9; 6.8	4.2‡	2.7; 5.7	4.3‡	2.8; 5.9	4.7‡	2.6; 6.8	4.3‡	2.2; 6.4	3.6†	1.5; 5.8	5.7‡	4.3; 7.1	4.2‡	2.7; 5.6	4.7‡	3.2; 6.2
2	5.2‡	3.7; 6.6	4.0‡	2.5; 5.5	4.1‡	2.6; 5.6	4.5‡	2.4; 6.6	4.1‡	2.0; 6.2	3.4†	1.3; 5.6	5.5‡	4.1; 6.9	4.0‡	2.5; 5.5	4.4‡	3.0; 5.9
3a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3b	4.6‡	3.1; 6.0	3.5‡	2.0; 5.0	3.6‡	2.1; 5.2	3.7†	1.6; 5.8	3.4†	1.3; 5.5	2.8†	0.7; 5.0	5.0‡	3.6; 6.5	3.6‡	2.1; 5.0	4.1‡	2.6; 5.6
3c	4.7‡	3.2; 6.1	3.7‡	2.2; 5.1	3.8‡	2.2; 5.3	4.0‡	1.9; 6.1	3.7†	1.6; 5.8	3.1†	1.0; 5.3	5.1‡	3.7; 6.4	3.7‡	2.2; 5.1	4.1‡	2.6; 5.6
3d	5.1‡	3.7; 6.6	4.0‡	2.5; 5.5	4.1‡	2.6; 5.6	4.5‡	2.4; 6.5	4.1‡	2.0; 6.2	3.4†	1.0; 5.3	5.5‡	4.1; 6.8	4.0‡	2.5; 5.5	4.5‡	3.0; 5.9
3e	4.9‡	3.5; 6.3	3.6‡	2.2; 5.1	3.9‡	2.4; 5.4	4.1‡	2.1; 6.2	3.5‡	1.5; 5.6	3.2†	1.0; 5.3	5.3‡	3.9; 6.6	3.7‡	2.2; 5.1	4.3‡	2.8; 5.7
3	4.1‡	2.6; 5.5	3.0‡	1.5; 4.5	3.3‡	1.8; 4.9	3.0†	0.9; 5.1	2.7†	0.6; 4.7	2.4*	0.3; 4.5	4.6‡	3.2; 6.0	3.2‡	1.7; 4.7	3.8‡	2.3; 5.3
4	1.7*	0.3; 3.1	1.3	-0.3; 2.7	1.3	-0.1; 2.7	0.0	-2.2; 2.2	0.6	-1.4; 2.7	0.4	0.3; 0.5	2.6‡	1.2; 3.9	1.7*	0.4; 3.1	2.0†	0.6; 3.5

	Body mass index (kg/m^2)						Sum of 4 skinfolds (mm)						Skinfolds ratio (*10)					
	T1 vs. T3		T1 vs. T3		T3 vs. T1		T1 vs. T3		T1 vs. T3		T3 vs. T1		T1 vs. T3		T1 vs. T3		T3 vs. T1	
	DC		CC		YEM		DC		CC		YEM		DC		CC		YEM	
Model	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
1	1.06‡	0.47; 1.65	0.69*	0.12; 1.25	0.98*	0.15; 1.24	6.1‡	2.9; 9.4	3.7†	0.8; 6.7	5.8‡	2.7; 8.9	0.36‡	0.22; 0.51	0.31‡	0.16; 0.46	0.26‡	0.11; 0.40
2	1.08†	0.46; 1.70	0.71*	0.12; 1.30	1.00†	0.40; 1.58	6.2‡	2.9; 9.6	3.8†	0.7; 6.8	5.7‡	2.6; 8.9	0.34‡	0.20; 0.49	0.29‡	0.14; 0.44	0.24†	0.10; 0.38

3a	0.70*	0.10; 1.30	0.41	-0.15; 0.97	0.69*	0.13; 1.25	3.9*	0.7; 7.2	2.0	-1.0; 4.9	3.9*	0.8; 7.0	0.28‡	0.14; 0.43	0.25‡	0.10; 0.40	0.20†	0.05; 0.34
3b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3c	0.77*	0.18; 1.36	0.51	-0.05; 1.08	0.76†	0.21; 1.31	4.3†	1.1; 7.5	2.5	-0.4; 5.4	4.2†	1.2; 7.2	0.27‡	0.14; 0.41	0.25‡	0.11; 0.40	0.20†	0.06; 0.33
3d	1.02†	0.41; 1.63	0.67*	0.08; 1.26	0.96†	0.37; 1.54	5.9†	2.6; 9.2	3.6†	0.5; 6.6	5.6‡	2.5; 8.8	0.34‡	0.20; 0.48	0.30‡	0.15; 0.45	0.24†	0.10; 0.38
3e	1.05†	0.43; 1.67	0.67*	0.08; 1.26	0.96†	0.39; 1.56	5.8†	2.5; 9.1	3.2*	0.2; 6.2	5.5†	2.4; 8.5	0.34‡	0.20; 0.49	0.30‡	0.15; 0.45	0.24†	0.10; 0.39
3	0.46	-0.11; 1.03	0.41	-0.09; 0.91	0.51	-0.02; 1.04	2.4	-0.7; 5.5	1.0	-1.9; 3.8	2.8	-0.2; 5.7	0.25‡	0.11; 0.38	0.24‡	0.10; 0.39	0.16*	0.03; 0.30
4	0.19	-0.42; 0.80	0.05	-0.25; 0.36	0.28	-0.27; 0.83	1.1	-2.1; 4.2	-1.3	-3.2; 0.5	1.6	-1.4; 4.7	0.29‡	0.15; 0.43	0.19†	0.08; 0.30	0.18†	0.04; 0.32

Model 1: adjusted for sex, height and time;
Model 2: model 1 + lifestyle variables (i.e., total energy intake, physical activity level, smoking and alcohol consumption status);
Model 3a: model 2 + MAP; Model 3b: model 2 + skinfolds ratio; Model 3c: model 2 + total-to-HDL cholesterol ratio; model 3d: model 2 + VO_2max; Model 3e: model 2 + resting heart rate;
Model 3: model 2 + all risk factors in models 3a-e;
Model 4: model 3 + current $MAP_{supine.}$
*p<0.05; †p<0.01; ‡p<0.001.
Note: columns shaded in grey show data also shown in Table 2 of printed manuscript and are also shown here to enable direct comparison across stiffness estimates.

Table S2. Mean differences in blood lipids and physical fitness, throughout the 24-year longitudinal period, between subjects with stiffer *vs.* less stiff carotid arteries at the age of 36, as defined on the basis the carotid DC, CC or YEM.

	Total-to-HDL cholesterol ratio						Triglycerides‖					
	T1 vs. T3 DC		T1 vs. T3 CC		T3 vs. T1 YEM		T1 vs. T3 DC		T1 vs. T3 CC		T3 vs. T1 YEM	
Model	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
1	0.37†	0.15; 0.58	0.23*	0.04; 0.43	0.27*	0.06; 0.48	1.18†	1.07; 1.30	1.12*	1.01; 1.23	1.13*	1.03; 1.24
2	0.34†	0.13; 0.55	0.22*	0.03; 0.42	0.24*	0.03; 0.44	1.19‡	1.08; 1.30	1.12*	1.01; 1.23	1.14†	1.03; 1.25
3a	0.25*	0.04; 0.46	0.15	-0.04; 0.34	0.16	-0.04; 0.37	1.13*	1.03; 1.24	1.09	0.99; 1.21	1.09	1.00; 1.20
3b	0.20	-0.00; 0.40	0.11	-0.08; 0.30	0.14	-0.06; 0.34	1.14†	1.04; 1.25	1.12*	1.01; 1.23	1.10*	1.00; 1.20
3c	-	-	-	-	-	-	-	-	-	-	-	-
3d	0.32†	0.11; 0.53	0.20*	0.01; 0.41	0.24*	0.03; 0.45	1.16†	1.05; 1.27	1.09	0.98; 1.20	1.11*	1.01; 1.22
3e	0.32†	0.11; 0.53	0.20*	0.01; 0.41	0.22*	0.01; 0.43	1.16†	1.05; 1.27	1.09	0.99; 1.21	1.12*	1.02; 1.23
3	0.11	-0.09; 0.31	0.10	-0.10; 0.29	0.09	-0.11; 0.28	1.05	0.96; 1.15	1.02	0.94; 1.12	1.02	0.94; 1.12
4	0.09	-0.12; 0.29	0.06	-0.13; 0.26	0.06	-0.13; 0.26	1.02	0.93; 1.12	1.00	0.91; 1.10	1.00	0.91; 1.10

	VO_2max (mL/min per kg^{FFM})						Resting heart rate (bpm)					
	T1 vs. T3 DC		T1 vs. T3 CC		T3 vs. T1 YEM		T1 vs. T3 DC		T1 vs. T3 CC		T3 vs. T1 YEM	
Model	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
1	-1.39*	-2.58; -0.19	-1.19*	-2.68; -0.09	-0.64	-1.84; 0.56	4.7‡	2.3; 7.1	6.0‡	3.7; 8.3	3.2†	1.0; 5.5
2	-1.28*	-2.44; -0.13	-1.21*	-2.35; -0.07	-0.50	-1.66; 0.66	4.2†	1.8; 6.6	5.6‡	3.3; 8.0	2.7*	0.5; 5.0
3a	-1.26*	-2.42; -0.10	-1.20*	-2.34; -0.05	-0.46	-1.62; 0.70	3.4†	1.0; 5.7	5.0‡	2.7; 7.3	2.0	-0.2; 4.3
3b	-1.45*	-2.61; -0.29	-1.27*	-2.48; -0.07	-0.60	-1.76; 0.56	4.2†	1.8; 6.6	5.6‡	3.3; 8.0	2.7*	0.4; 5.0
3c	-1.01	-2.17; 0.14	-0.98	-2.20; 0.24	-0.32	-1.49; 0.85	3.8†	1.3; 6.2	5.4‡	3.0; 7.7	2.3*	0.1; 4.6
3d	-	-	-	-	-	-	3.8†	1.4; 6.1	5.3‡	3.0; 7.6	2.5*	0.3; 4.7
3e	-0.96	-2.09; 0.17	-0.93	-2.06; 0.20	-0.28	-1.43; 0.86	-	-	-	-	-	-
3	-1.03	-2.17; -0.11	-0.83	-2.03; 0.37	-0.34	-1.51; 0.83	2.9*	0.5; 5.2	4.7‡	2.5; 7.0	1.7	-0.5; 3.9
4	-0.74	-1.98; 0.51	-0.66	-1.80; 0.49	-0.04	-1.27; 1.20	2.3	-0.2; 4.9	4.4‡	2.1; 6.7	1.0	-1.3; 3.4

Model 1: adjusted for sex, height and time;
Model 2: model 1 + lifestyle variables (i.e., total energy intake, physical activity level, smoking and alcohol consumption status);
Model 3a: model 2 + MAP; Model 3b: model 2 + skinfolds ratio; Model 3c: model 2 + total-to-HDL cholesterol ratio; model 3d: model 2 + VO_2max; Model 3e: model 2 + resting heart rate;
Model 3: model 2 + all risk factors in models 3a-e;
Model 4: model 3 + current MAP_{supine}.
*p<0.05; †p<0.01; ‡p<0.001.
Note: columns shaded in grey show data that are also described in text of printed manuscript and are also shown here to enable direct comparison across stiffness estimates.